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Repeated social stress leads to contrasting patterns of structural plasticity in the amygdala and hippocampus

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Repeated social stress in rats leads to contrasting patterns of structural plasticity in the amygdala and hippocampus

D. Patel, S. Anilkumar, S. Chattarji and B. Buwalda

Abstract

Previous studies have demonstrated that repeated immobilization and restraint stress cause contrasting patterns of dendritic reorganization as well as alterations in spine density in amygdalar and hippocampal neurons. Whether social and ethologically relevant stressors can induce similar patterns of morphological plasticity remains largely unexplored. Hence, we assessed the effects of repeated social defeat stress on neuronal morphology in basolateral amygdala (BLA), hippocampal CA1 and infralimbic medial prefrontal cortex (mPFC). Male Wistar rats experienced social defeat stress on 5 consecutive days during confrontation in the resident-intruder paradigm with larger and aggressive Wild-type Groningen rats. This resulted in clear social avoidance behavior one day after the last confrontation. To assess the morphological consequences of repeated social defeat, 2 weeks after the last defeat, animals were sacrificed and brains were stained using a Golgi-Cox procedure. Morphometric analyses revealed that, compared to controls, defeated Wistar rats showed apical dendritic decrease in spine density on CA1 but not BLA. Sholl analysis demonstrated a significant dendritic atrophy of CA1 basal dendrites in defeated animals. In contrast, basal dendrites of BLA pyramidal neurons exhibited enhanced dendritic arborization in defeated animals. Social stress failed to induce lasting structural changes in mPFC neurons. Our findings demonstrate for the first time that social defeat stress elicits divergent patterns of structural plasticity in the hippocampus versus amygdala, similar to what has previously been reported with repeated physical stressors. Therefore, brain region specific variations may be a universal feature of stress-induced plasticity that is shared by both physical and social stressors.

Keywords: social defeat stress; social avoidance behavior; CA1; BLA; mPFC; Golgi-cox

Introduction

Growing evidence has suggested that stress induced by adverse experiences may lead to acute as well as long lasting changes at multiple levels of neural organization [1–3]. The adult brain is known to possess remarkable structural plasticity in response to stress exposure [1,4]. Stress-induced structural remodeling of neuronal architecture is an attempt to adapt to the stressor. Failing to do so may contribute to the onset and recurrence of mood disorders like depression and anxiety [5,6].

Three brain regions known to mediate stress by differentially regulating the hypothalamus-pituitary-adrenal (HPA) axis are the hippocampus, amygdala and prefrontal cortex [1,7,8]. From clinical and neuroimaging studies in humans, these brain regions have been established to undergo functional and structural changes with stress disorders [9,10]. Moreover, studies suggest that impairments in the structural plasticity and volumetric changes of specific limbic areas contribute to the pathophysiology of mood and major depressive disorders [11–13].

Focusing on the rat brain, evidence from several studies have established that repeated or chronic stress causes opposite patterns of morphological plasticity in the amygdala versus hippocampus. For instance, McEwen and colleagues showed remarkable dendritic atrophy occurring in the pyramidal neurons of the CA3 subregion of the hippocampus after 21 days of chronic restraint stress (CRS). Similarly, 10 days of chronic immobilization stress (CIS) and other restraint stress models have shown to induce shrinkage in hippocampal CA3 neurons, marked by decreased branching and a reduction in the length of the apical dendrites [14–16]. Similarly, the prefrontal cortex shows a dendritic atrophy in response to immobilization stress [17–19]. In the amygdala, chronic immobilization stress (CIS) is known to induce an opposite structural change with dendritic hypertrophy in the pyramidal and stellate neurons [16].

Chronic stress not only causes dendritic remodeling but also changes in spine shape and density. Various physical stressors decrease the spine density in the CA3 and the CA1 pyramidal neurons, hence associating it with depression-like behaviors observed in animal models [20–22]. Moreover, spine loss is also observed in the apical dendrites of pyramidal medial prefrontal cortex (mPFC) neurons in male rats subjected to chronic restraint stress [23,24]. In contrast, CIS and acute immobilization stress (AIS) are also known to enhance spinogenesis across both primary and secondary branches of spiny neurons in the BLA where AIS induces gradual formation of new spines over time but without any effect on dendritic arbors [25].

All these past studies relied on repeated exposures to severe physical stressors, such as 2h/day immobilization for 10 days or restraint for 6h/day for 21 days. As useful as these models have been in elucidating various facets of stress effects on the brain, their ethologically relevance is limited and does not capture the uniquely species specific social or psychological nature of

69 stress. Indeed, whether or not such social stressors also trigger divergent patterns of plasticity in
70 the amygdala and hippocampus is not known. The present study aims to bridge this gap in
71 knowledge.

72
73 The most frequently used paradigm to study social stress in rodents is the experience of a defeat
74 during an aggressive encounter in the resident-intruder paradigm. For the above reasons, it was
75 hypothesized that manipulating the social environment of Wistar rats by subjecting them to
76 repeated social stress of defeat, would alter behavior as well as the neuronal morphology of
77 hippocampal, amygdalar and prefrontal brain regions, in particular the CA1, BLA, and mPFC,
78 involved in emotional and cognitive performance. If corticosterone secretion due to the stress
79 exposure is playing an important role in structural remodeling in these brain regions [26], we
80 expect that, on the basis of similarity of the neuroendocrine response to immobility and social
81 defeat stress [27], the changes will be similar in both stress paradigms. It may be, however, that
82 temporal dynamics of the changes in brain regions are dissimilar [28].

1. Materials and methods

2.1. Experimental Animals

Male Wistar rats (Harlan, the Netherlands) were used as experimental animals and Wild-type Groningen (WTG) rats as residential males. Wistar rats (four months old and weighing 350-400g at the beginning of the experiment) were singly housed following the first social defeat experience. Residential WTG males were around six months old. The animals were kept with 12/12-hour reversed light/dark cycle (lights off at 10:00h) and food and water was given ad libitum. All behavioral experimental procedures were performed during the dark phase (11:00-15:00h) of the cycle. All experimental protocols conducted were approved by the Animal Ethics Committee of Groningen University.

2.2. Experimental Design

2.2.1. Social Stress Protocol (Resident- intruder paradigm and psychosocial threat)

Wistar rats from the social stress group were subjected to social defeats using the resident-intruder paradigm and intermittently exposed to psychosocial threat (see Fig. 1). In the resident-intruder paradigm, (see Fig. 1 box B.) male Wistar rats (intruders) were placed in the cage (80×55×40 cm) of an aggressive WTG male rat (resident). The resident rat was housed in a large cage (80x55x40 cm) with a female wild-type rat to evoke territorial aggression. One-hour prior to the defeat, the female was removed from the resident's cage. Rats from the social stress group were exposed to the residents three times (day 1,2 and 4) for 10 min allowing direct physical contact. After 10 min, the intruder experimental rat was placed in a wire mesh cage (14x14x24 cm) for 50 minutes in the resident's cage allowing psychosocial threat of attack but protecting it from severe physical injuries. Subsequently, Wistar intruders were returned to the home cage (singly housed). On the 3rd and 7th day, the defeated intruder rats were directly placed in the protective wire mesh cage (14×14×24 cm) and introduced into the resident's cage (see Fig. 1 box C.). Control Wistar rats were placed in an empty residential cage. Following the defeat or control treatment, all experimental Wistar rats were singly housed. Body weights of all the experimental rats were noted prior, during and post treatment (see Fig. 2) of the protocol. Based upon the quality of the defeat (vocalization and submissive postures during aggressive encounters and impact of defeat on body weight gain) 6 animals were short-listed out of 13 for the study of the impact of social stress on structural remodeling. These rats showed the strongest behavioral and body weight response to the defeat exposure. In the total group of 13 stressed rats body weight gain over the first week was 20 ± 1.3 g in controls versus -3 ± 2.9 g in stressed rats. In the selected group of 6 rats it was 19 ± 2.3 g in controls versus -10 ± 3.9 g in stressed rats. Six randomly chosen control animals were matched for Golgi analysis.

2.2.2. Experiment 1. Effects of repeated social stress on Social avoidance behavior:

The control (N=6) and socially stressed (N=6) animals were behaviorally tested on day 0 (a day before the onset of first social defeat) and 8th day of the protocol. The social avoidance behavior

was performed in a 1x1 m open arena. An unfamiliar WTG male was enclosed in a wire mesh cage as a social stimulus and this was located on the sidewall of the arena. An experimental intruder rat was then introduced into the arena at the opposite side of the WTG male kept in the wire mesh cage. The intruder rat was allowed to freely explore the cage for 3 min. Behavior was recorded with a video camera and analyzed for different parameters such as time spent in interaction zone (sec), latency to enter interaction zone (sec), the frequency of entering interaction zone and total distance traveled (cm) in the cage by the experimental animal (Fig. 1 box D).

2.2.3. Experiment 2. Long-lasting effects of repeated social stress on morphology of the amygdala, hippocampal and prefrontal cortical neurons.

2.2.3.1. Modified Golgi-Cox staining:

On 22nd day of the protocol, which was 2 weeks after the last social stress experience, all experimental animals were sacrificed via rapid decapitation. Brains were removed and dropped in Golgi-Cox fixative. After 15 days of incubation at room temperature in the Golgi-cox fixative, 120 μ m thick coronal sections were obtained using a fixed tissue vibratome (Leica VT 1200S). Sections were serially collected, the color was developed by sodium carbonate and subsequently the brain sections were dehydrated in absolute alcohol, cleared in xylene and cover-slipped (as slightly adapted from [29]). Prior to quantitative analysis, slides were coded and the experimenter was blind to the code. The codes were broken only after the morphological analysis was completed.

2.2.3.2. Morphological analysis:

On the basis of morphological criterion reported in [25] pyramidal neurons from the BLA region of the amygdala, CA1 region of the hippocampus and infralimbic region of the prefrontal cortex were selected. For morphological quantification, 5-8 pyramidal neurons from each animal (6 animals per group) were analyzed. The analysis of BLA, CA1 and mPFC neurons is restricted to those located within bregma -1.92 to -2.64mm, -2.40 to -3.96mm and 3.7 to 2.7mm respectively.

Analysis of dendritic arborization:

Morphometric analysis of dendritic arborization was done using the NeuroLucida software (Micro-BrightField, Williston, VT, USA) along with an Olympus BX61 microscope (40X, 0.75 numerical aperture, Olympus BX61; Olympus, Shinjuku-Ku, Tokyo, Japan). Starting from the centre of the soma (as a reference point), two parameters (number of interactions and the dendritic length) were measured as a function of radial distance from the soma by adding up all values in each successive concentric segment (Sholl's analysis; starting radius and radius increment: 10 μ m for BLA pyramidal-like neurons and 20 μ m for CA1 and mPFC pyramidal neurons) [16].

Analysis of dendritic spine density:

For the analysis of dendritic spine density, the same NeuroLucida software attached to an Olympus BX61 microscope (100X, 1.3 numerical aperture, Olympus BX61; Olympus, Shinjuku-Ku, Tokyo, Japan) was used. The dendrites directly originating from the main shaft are classified as primary apical dendrites which were used for the primary apical dendrite spine quantification. However, secondary basal dendrites emerging from the primary basal dendrite were used for the spine density analysis (Fig. 4). Starting from the origin of the branch, and continuing away from the cell soma, spines were counted manually along 80 μm stretch of the selected dendrite. Furthermore, this spine density analysis was done using a detailed segmental analysis. The segmental analysis consisted of counting the number of spines in successive steps of 10 μm each, for a total of 8 steps (i.e. a total length of 80 μm). The values for each segment, at a given distance from the origin of the branch, were then averaged across all neurons in the experimental group [25].

2.3. Statistical Analysis

Statistical significance was calculated using Student's t-test. In the morphological analysis n -values refer to the number of dendrites (spine-density analysis) and also number of cells (Sholl analysis). However, capital N refers to the number of animals used. All the behavioral parameters along with the change in body weight gain and all the morphological segmental data were analyzed using repeated measures two-way ANOVA and post hoc Bonferroni test was used for multiple comparisons with significance levels set at $p < 0.05$. The factors for the behavioral test were: social defeat (control vs social stress) and days (0 and 8); for spine density analysis: social defeat (control vs social stress) and distance from the origin from the branch (10-80 μm); and for Sholl analysis: social defeat (control vs social stress) and radius [0 to 240 μm (apical dendrites)/160 μm (basal dendrites)]. For the bar plots, unpaired t-test was used to compare between social stress and control groups. For correlation analysis Pearson's r was calculated along with the p -value. Statistical analyses were performed using Prism 6 (GraphPad software). Significance was set at $p < 0.05$ for all analyses and values were reported as mean \pm s.e.m (standard error of the mean).

2. Results

3.1 Effect of repeated social stress on body weight.

We first studied the impact of the social defeat stress on the body weight gain comparing the group of animals subjected to social stress and the controls (unstressed group) across the period of the experimental plan (detailed in methods). We found that socially stressed rats show significantly reduced body weights compared to the control rats from day 5 to day 23 (mean \pm s.e.m). (Factor stress: $F(1, 10) = 47.24, p < 0.0001$; Factor days: $F(18, 180) = 246.3, p < 0.0001$; interaction: $F(18, 180) = 31.74, p < 0.0001$) (Fig. 2).

3.2 Effect of repeated social stress on social avoidance behavior.

Social stress induces social anxiety, which was tested by social avoidance behavior, and the behavior was compared at day 0 and day 8. Socially stressed rats (N=6) showed significant social avoidance behavior (mean \pm s.e.m), in the presence of an encaged WTG rat as a social stimulus, when compared with control (N=6) rats (Fig. 3).

3.2.1 Time spent in the interaction zone (sec)

Stressed Wistar rats spent significantly less time in the interaction zone exploring the social stimuli compared to control Wistar rats (Factor stress: $F(1, 10) = 14.00, p = 0.0038$; Factor days: $F(1, 10) = 1.364, p = 0.2699$; interaction: $F(1, 10) = 11.17, p = 0.0075$). Further analysis using *post-hoc* Bonferroni's test for multiple comparisons indicated that unstressed Wistar rats spent significantly more time exploring the stimulus compared to the socially stressed rats on day 8 ($p < 0.001$). This suggests that Wistar rats, that were socially defeated, showed inhibition in exploring the social stimulus rat whereas the control rats showed enhanced social behavior (Fig. 3 A).

3.2.2 Number of entries in the interaction zone

The significant difference was seen in the interaction between factors: time and stress as indicated by two-way ANOVA (Factor stress: $F(1, 10) = 11.96, p = 0.0061$; Factor days: $F(1, 10) = 0.04210, p = 0.8415$; interaction: $F(1, 10) = 7.115, p = 0.0236$). Socially stressed Wistar rats approached significantly less frequently to the social stimulus in comparison to the control Wistar rats on day 8 ($p < 0.001$). However, control rats showed a trend of increased frequency of visits to the interaction zone from day 0 to day 8. On the other hand, socially defeated rats showed a decrease in trend from day 0 to day 8 in the number of times for exploring the social stimulus (Fig. 3 B).

3.2.3 Latency to enter in the interaction zone (sec)

There was a significant difference found in the factor stress ($F(1, 10) = 12.90, p = 0.0049$) by ANOVA where socially stressed rats showed higher latency to enter in the interaction zone in comparison to the control on day 8 ($p < 0.01$). No difference was found between day 0 and day 8

for both the groups. Instead, it appears that the defeated rats initially were reluctant to visit the encaged social stimulus (Fig. 3 C).

3.2.4 Total Distance travelled (cm)

Socially defeated rats travelled less in the arena with the encaged social stimulus in comparison with the control rats ($F(1, 10) = 5.319, p = 0.0438$) (Fig. 3 D).

3.3. Longer-term effects of repeated social stress on the morphology of hippocampus (CA1) and the amygdala (BLA) neurons.

The Golgi-cox impregnation is considered to be a well-established procedure for clearly identifying the region of interest in the CA1, BLA and mPFC areas and studying the neuronal morphology and dendritic spine phenotype in these structures. The number of animals (N) used for this experiment is 6 in each group (control and socially stressed Wistar rats).

3.3.1. Effects of social stress on pyramidal neurons of the CA1 region of the hippocampus:

The analysis of the number of intersections and dendritic length revealed alterations in the basal but not in the apical dendritic morphometry of the socially defeated animals (Fig. 5 A, C-D, G-H, K-L, O-P). In socially stressed rats both the number of basal dendritic intersections ($F(1, 10) = 9.558, p = 0.0114$) as well as basal dendritic length ($F(1, 10) = 9.403, p = 0.0119$) are reduced as compared to controls. Further investigation using *post-hoc* analysis revealed that decreased dendritic arborization in the basal dendrites was due to reduction in the number of intersections as well as dendritic length. Reduction was seen particularly at a radial distance of 80 μ m and 100 μ m, in the number of intersections (Fig. 5 K-L) and from 80 μ m to 120 μ m from the soma in the dendritic length (Fig. 5 O-P, unpaired t-test * $p < 0.05$) in the basal dendrites.

In terms of spine density, the social stress influenced the number of spines in the hippocampal CA1 area (Fig. 7 A-F). Socially stressed Wistar rats had significantly lower spine density in CA1 in the apical dendrites (Fig. 7 A-C) ($F(1, 10) = 64.66, p < 0.0001$) but not in basal dendrites (Fig. 7 D-F) ($F(1, 10) = 2.058, p = 0.1820$) in comparison with the neurons in the control rats. *Post-hoc* testing revealed a stress-induced reduction in the spine density in the apical dendrites at 10 μ m, 30 μ m, 60-80 μ m distance from origin of branch (Fig. 7 A).

3.3.2. Effects of social stress on pyramidal neurons of the BLA region of the Amygdala:

As shown in Fig. 5 B, E-F, I-J, M-N, Q-R, repeated social stress induces elongation of basal dendrites of the BLA pyramidal neurons and does not affect apical dendrites in the same pyramidal neurons. Analysis by two-way ANOVA with stress and Sholl radius as variables and the interaction between these two revealed a significant increase in the number of basal dendritic intersections ($F(1, 10) = 8.009, p = 0.0179$) and dendritic length ($F(1, 10) = 10.06, p = 0.0099$) in stressed rats as compared to controls. There was no significant difference in the interaction of both the factors. Further, *post-hoc* analysis and unpaired t-test (Fig. 5 N, R) revealed that socially

stressed rats display significantly more number of intersections in at a radial distance of 40µm, 60µm and 70µm from the soma and increase in dendritic length at a radial distance of 50µm to 70µm (Fig. 5 M, Q). No differences, in either number of intersections and total dendritic length, were noted in the apical dendrites of the pyramidal neurons of the socially stressed versus control Wistar rats (Fig. 5 E-F, I-J).

Statistical analysis revealed no significant difference in spine density of the BLA pyramidal neurons for both apical (Fig.7 G-I) ($F(1, 10) = 3.003, p = 0.1138$) and basal (Fig.7 J-L) ($F(1, 10) = 1.609, p = 0.2333$) primary dendrites.

3.3.3. Effects of social stress on pyramidal neurons of the infralimbic region of the medial Prefrontal cortex:

As shown in Fig. 6 repeated social stress did not affect apical and basal dendrites of the mPFC pyramidal neurons as indicated by two-way ANOVA analysis. Also apical or basal dendritic spines were not affected by the stress exposure (data not shown).

3.4. Correlations between social anxiety induced by social avoidance behavior and morphological measurements.

Results from controls and socially stressed rats were correlated for all behavioral and morphological measurements. We found significant correlation between the number of visits in interaction zone with basal dendritic arborization of BLA and CA1 neurons and apical dendritic spines of CA1 pyramidal neurons. There was no significant correlation between behavioral parameters with other morphological measurements (for $N = 12$). Results revealed that neuronal morphology in CA1 correlates with the behavior, showing that animals with stronger avoidance show stronger reduction in apical dendritic spine density (Pearson's $r = 0.7892, p = 0.0023$; Fig. 8. A) and basal dendritic atrophy (For number of intersections: Pearson's $r = 0.5875, p = 0.0446$; for dendritic length: Pearson's $r = 0.5964, p = 0.0407$; Fig. 8. B and C). However, in BLA only basal dendrites (hypertrophy) are significantly correlated with social avoidance behavior (for number of intersections: Pearson's $r = -0.6597, p = 0.0196$; for dendritic length: Pearson's $r = -0.6420, p = 0.0244$; Fig. 8. D and E).

3. Discussion

The results in this study indicate that social anxiety in rats following repeated social defeat exposure is combined with divergent structural remodeling of dendrites in amygdalar and hippocampal neurons. This extends the previous findings in non-social restraint and immobilization stress to ethologically relevant social stress models. A week with 5 daily exposures to social defeat stress or psychosocial threat of attack increased social avoidance behavior 1 day after the last stress exposure which correlated significantly with the changes in structural morphology two weeks later of BLA and CA1 pyramidal neurons. The correlations are largely visualizing the group differences in social avoidance behavior and structural alterations. In hippocampal CA1 neurons a significant loss in spines in apical dendrites as well as dendritic atrophy in basal dendrites was observed. Basal dendrites in BLA pyramidal neurons showed dendritic hypertrophy. In the infralimbic mPFC no lasting consequences of social defeat stress were measured with regards to dendritic and spine remodeling.

In the hippocampus particularly the CA3 region was reported to be sensitive to chronic glucocorticoid treatment [30] and therefore quite a number of studies focused on structural remodeling following stress in that brain region. It was shown that chronic stress leads to dendritic atrophy in the hippocampal CA3 pyramidal neurons, marked by decreased branching and a reduction in the length of the apical dendrites [14–16,31]. Structural remodeling in the CA1 region, which serves as one of the major output structures of the hippocampal formation, was less frequently studied but moves in a similar direction. Prolonged activity-induced stress or corticosterone administration was reported to cause dendritic retraction of CA1 pyramidal neurons [32,33].

In our experiments we see that following social stress, dendritic atrophy in CA1 neurons is rather robust and persistent after two weeks of recovery. A number of studies indicate that dendritic atrophy of the CA3 pyramidal neurons has a transient character and reverses within 21 days [34–36]. However, in other studies more persistent hippocampal atrophy is observed. In a human post-mortem study persistent dendritic atrophy of hippocampal CA3 neurons is reported in subjects undergoing severe psychological stress [37]. Lasting structural effects in the CA3 pyramidal neurons were also seen three weeks after a double social defeat in rats [38]. This particular study showed a striking difference in the temporal dynamics of structural remodeling immediately after a three week period of intermittent defeats and after a three week delay following two defeats on subsequent days. Three weeks after a double defeat a significant increase was found in CA3 basal dendrite surface whereas 1 day after a three week period of social stress every other day a significant decrease in both basal and apical dendritic surface was observed [38]. The mechanisms underlying dendritic remodeling in CA3 pyramidal neurons after chronic social stress are likely to be mediated by stress-induced changes in glucocorticoids

[5,15,39]. As mentioned above this region of the hippocampus shows to be particularly vulnerable to high corticosteroid levels [30].

In contrast to the general stress-induced atrophy of the dendritic tree in the hippocampus, principal neurons in the BLA exhibit dendritic hypertrophy 24 hours after chronic immobilization stress (CIS), which persists even after three weeks of stress free recovery. This robust dendritic hypertrophy induced by CIS in the BLA is accompanied by greater anxiety-like behavior in the animals [16,36]. Both physical and psychosocial stressors are known to increase anxiety in rodents. Lesioning the amygdala blocks this stress-induced increase in anxiety [40] indicating the link between amygdalar hypertrophy following stress exposure and anxiety. In our experiment acute effects of social defeat experience were visualized in the increased social avoidance in defeated animals.

Stress not only causes changes in the arborization of the dendritic tree but also alters the synaptic connectivity by changing spine shape and density. Various physical stressors decrease the spine density in the CA3 and the CA1 pyramidal neurons, associating it with depressive like behaviors observed in the animals [20–22]. Both chronic (CIS) and acute immobilization stress (AIS) are also known to enhance spinogenesis in the BLA pyramidal neurons [25,29]. AIS induces gradual formation of new spines over time without any effect on dendritic arbors. Interestingly, the delayed generation of spines after AIS was accompanied by a gradual development of anxiety like behavior in rodents. This study shows that higher anxiety in rodents can arise due to BLA spinogenesis in the absence of dendritic hypertrophy [25]. On the other hand, a study from Mitra and Sapolsky showed that a single acute dose of corticosterone was sufficient to induce dendritic hypertrophy in the BLA and also elevated levels in anxiety in rats, when measured on an elevated plus maze [41].

We anticipated a reduction of dendrites and/or spines in neurons of the infralimbic mPFC since this brain region has proven to be sensitive to the remodeling potential of restraint stress. Somewhat unexpected, social defeat stress did not cause alterations in dendrites and spines in neurons of the infralimbic mPFC two weeks after the last defeat exposure. It is possible that temporal dynamics in the structural alterations in different brain regions are playing a role in this finding. The dendritic remodeling following acute corticosterone administration was delayed in the basolateral amygdala as compared to that in neurons of the mPFC [28]. It is possible that remodeling in the mPFC already recovered after stress exposure.

Evidence from previous studies consistently shows that repeated social defeat in both mice and rats elicits social avoidance behavior [42,43]. We previously showed that more than a month after a social defeat experience, rats were showing social anxiety towards residential males [2]. The study performed by Vidal et al. [44] demonstrated that social defeat stress during adolescence (postnatal day 45–58) induced social avoidance behavior even up to 7 weeks later. These studies show that repeated exposure to social defeat stress consistently results in long

382 lasting social avoidance behavior, which acts as an indicator to measure social anxiety in
383 animals. This in contrast with the relatively short-lasting effects of social defeat stress on general
384 anxiety as reflected in the avoidance of the open arms of the elevated plus-maze [2]. Only a few
385 animals were persistently anxious in this test. This indicates that whereas social stressors long-
386 lastingly increase social anxiety, a substantial individual variation is observed in the vulnerability
387 to develop stress-induced general anxiety.

388
389 Neurotrophins such as brain derived neurotrophic factor (BDNF) are known to mediate
390 hippocampal dendritic and spine plasticity after chronic stress. Levels of BDNF expression in the
391 hippocampal CA3 and BLA reflected the opposing effect of CIS and AIS on structural
392 remodeling in these two brain regions [45]. Reports on the effects of social defeat stress on
393 hippocampal and amygdalar BDNF expression or protein levels are less clear. Social defeat
394 exposure in rats was reported to elicit a transient decrease in BDNF expression 24 hours later in
395 all hippocampal brain regions as well as the BLA [46]. Five and 14 days after the defeat, BDNF
396 expression returned to baseline levels. A study in hamsters reported, however, an increase in
397 amygdalar BDNF two hours after defeat without an effect in the hippocampus [47]. In defeated
398 mice increased expression of mature BDNF in the BLA was reported which proved to be
399 essential for the social avoidance behavior 24 hours later [48]. Studies have also shown that
400 transgenic overexpression of BDNF in mice has antidepressant effects and prevents hippocampal
401 atrophy induced by chronic stress providing genetic evidence linking structural plasticity in the
402 hippocampus with depressive like behavior. BLA overexpression of BDNF in transgenic mice is
403 known to cause spinogenesis and also leads to increased anxiety in the genetically engineered
404 mice [49].

405
406 The basal and proximal apical dendrites of CA1 pyramidal cells are known to receive input
407 primarily from CA3 cells [50]. In a study from Ghosh et. al., 2013 functional connectivity
408 between CA3, CA1 and BLA neurons was studied using electrophysiology after CIS. A
409 statistical analysis on time-series data, Granger causality, was used to study functional
410 interactions between these regions. This revealed a strong directional influence from the lateral
411 amygdala to the CA1 region that occurred during and lasted till even 10 days after chronic stress.
412 In contrast, the directional coupling from hippocampal CA3 region to CA1 gradually weakened
413 during and actually was absent 10 days after stress exposure [51]. These statistical relationships
414 as indicated by the Granger causality suggest that the persistent influence of the BLA on CA1
415 neuronal activity might explain the loss of spines in apical dendrites and dendritic atrophy in
416 basal dendrites of CA1 pyramidal neurons. A future challenge would be to study if indeed
417 structural changes in BLA neurons precede and cause hippocampal atrophy in CA1 dendritic
418 architecture and spine density. Findings in preclinical studies like the present one are largely
419 fundamental in nature. We expect, however, that this approach will ultimately contribute to a
420 better understanding and treatment of the behavioral deficits induced by chronic stress exposure
421 in humans.

422

423

Legends to the figures

Figure 1. Experimental plan followed for the study. (A) Each colored vertical bar represents a single day on which the particular experimental procedure was performed. Red, grey and black bars represent days on which respectively (B) stress of social defeat (C) psychosocial threat of defeat and (D) social avoidance behaviour were performed.

Figure 2. Body weight gain with time. Body weight gain is impaired in the socially stressed animals. The body weight (normalized) of the defeated rats ($N = 6$) is significantly lower than the control rats ($N = 6$) from day 4 to day 22 (mean \pm s.e.m). Asterisks indicate significant differences ($* p < 0.05$ level, Bonferroni's test for multiple comparisons).

Figure 3. Social stress enhances social anxiety behavior as measured using social avoidance behaviour. The plots represent quantification of behavior during social avoidance testing. Bars represent mean \pm s.e.m. Individual data are scatter plotted. (A) Time spent in the interaction zone, (B) Number of entries in the interaction zone, (C) Latency to enter in the interaction zone and (D) Total path length travelled. Asterisks indicate significant differences ($* p < 0.05$, $** p < 0.01$, $*** p < 0.001$, *post-hoc* Bonferroni's multiple comparison test).

Figure 4. (A) Low-power photomicrograph of a Golgi stain-impregnated pyramidal neuron in the BLA (scale bar, $20\mu\text{m}$). (inset) High-power image of spines on an apical and basal dendrite from the same neuron. (B) Schematic drawing classifying types of primary dendrites selected for spine density analysis. In our analysis, a dendritic branch emanating directly from the cell soma was defined as a main shaft, whereas a dendrite originating from a main shaft was defined as a primary branch. Spines were counted starting from the origin of a branch, in 10 consecutive segments of $8\mu\text{m}$ each.

Figure 5. Long term effects of social stress induced dendritic atrophy in CA1 pyramidal neurons but enhanced dendritic arborization in the basal dendrites of BLA pyramidal neurons. (A, B): Representative tracing of Golgi-impregnated pyramidal neurons in the CA1 (left two columns) and BLA (right two columns) region (scale bar, $10\mu\text{m}$). (C, G, K, O (CA1) and E, I, M, Q (BLA)): Effects of social stress on mean number of intersections and dendritic length for each successive $20\mu\text{m}$ segment as a function of the radial distance of the corresponding segment from the soma. (D, H, L, P (CA1) and F, J, N, R (BLA)): Mean total number of intersections and total dendritic length. For each group $N = 6$ rats and number of neurons ($n = 28$ for control, $n = 26$ for SS group) in CA1 brain region is shown. From BLA region, data from $n = 29$ neurons for control and $n = 32$ for SS is shown. Error bars expressed as mean \pm s.e.m. Hashtags indicate significant differences in segmental plots ($\# p < 0.05$, $\#\# p < 0.01$, $\#\#\# p < 0.001$ level, Bonferroni's test for multiple comparisons). An asterisk indicates significant differences in bar plots ($* p < 0.05$ level, unpaired t-test).

Figure 6. No long term effects of social stress on infralimbic mPFC pyramidal neurons (A): Representative tracing of Golgi-impregnated pyramidal neurons in the mPFC region (scale bar, 10 μ m). (B, D, F, H): Effects of social stress on mean number of intersections and dendritic length for each successive 20 μ m segment as a function of the radial distance of the corresponding segment from the soma. (C, E, G, I): Mean total number of intersections and total dendritic length. For each group N= 6 rats and number of neurons n= 37 for control, n= 36 for SS group in IL brain region is shown. Error bars expressed as mean \pm s.e.m. ns means not significant.

Figure 7. Long term effects of social stress decreases spine density in the apical dendrites of the CA1 pyramidal neurons. (C, F, I, L) Photomicrographs of representative segments of primary dendritic branches from neurons in controls (left) and social stress (right) (scale bar, 5 μ m). (A, D, G, J) Segmental analysis of mean numbers of spines in each successive 10 μ m segment of the 80 μ m primary dendrite as a function of the distance of that segment from the origin of the main shaft. (B, E, H, K) Mean values for spine-density (calculated as the average number of spines per 80 μ m of primary branches) for control (N= 6 animals; CA1 apical dendrites n= 39, basal dendrites n= 45; BLA apical dendrites n= 35 and basal dendrites n= 30) and socially stress (N= 6 animals; CA1 apical dendrites n= 40, basal dendrites n= 43; BLA apical dendrites n= 33 and basal dendrites n= 50). Error bars expressed as mean \pm s.e.m. Changes in CA1 (upper) and BLA (bottom) dendrites are shown separately. The panels containing images A,B,C and G,H,I depicts data from apical dendrites likewise D,E,F and J,K,L from basal dendrites from CA1 and BLA pyramidal neurons. Hashtags indicate significant differences in segmental plots (# $p < 0.05$, ## $p < 0.01$, ##### $p < 0.0001$ level, Bonferroni's test for multiple comparisons). Asterisks indicate significant differences in bar plots (**** $p < 0.0001$ level, unpaired t-test).

Figure 8. Correlation analysis for morphological measurements and behavioral parameters. Scatter plots illustrating the variation of number of visits in the interaction zone with different morphological parameters: spine density in CA1 (A), number of intersections in CA1 (B), total dendritic length (C), number of intersections in BLA (D) and total dendritic length in BLA (E). The values are the Pearson's r calculated for the pair of morphological measurement and behavioral parameter. An asterisk indicates significant differences with p values mentioned in brackets (* $p < 0.05$, ** $p < 0.01$).

498 Fig. 1

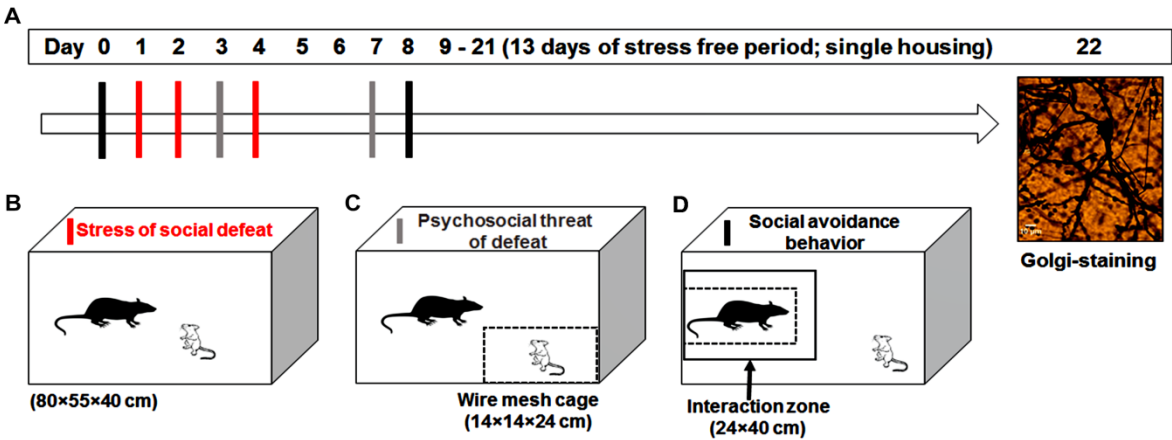
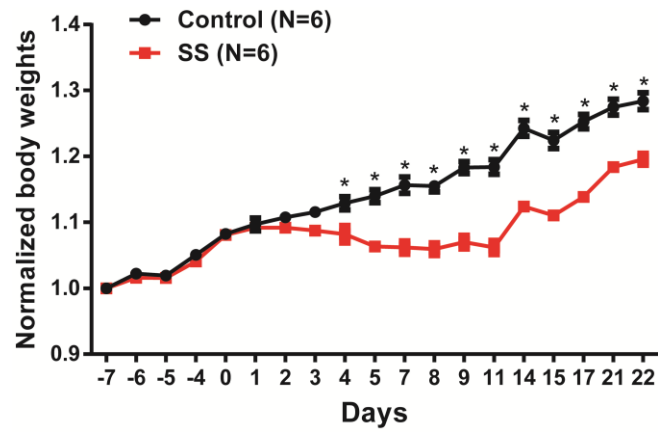


Fig. 2.



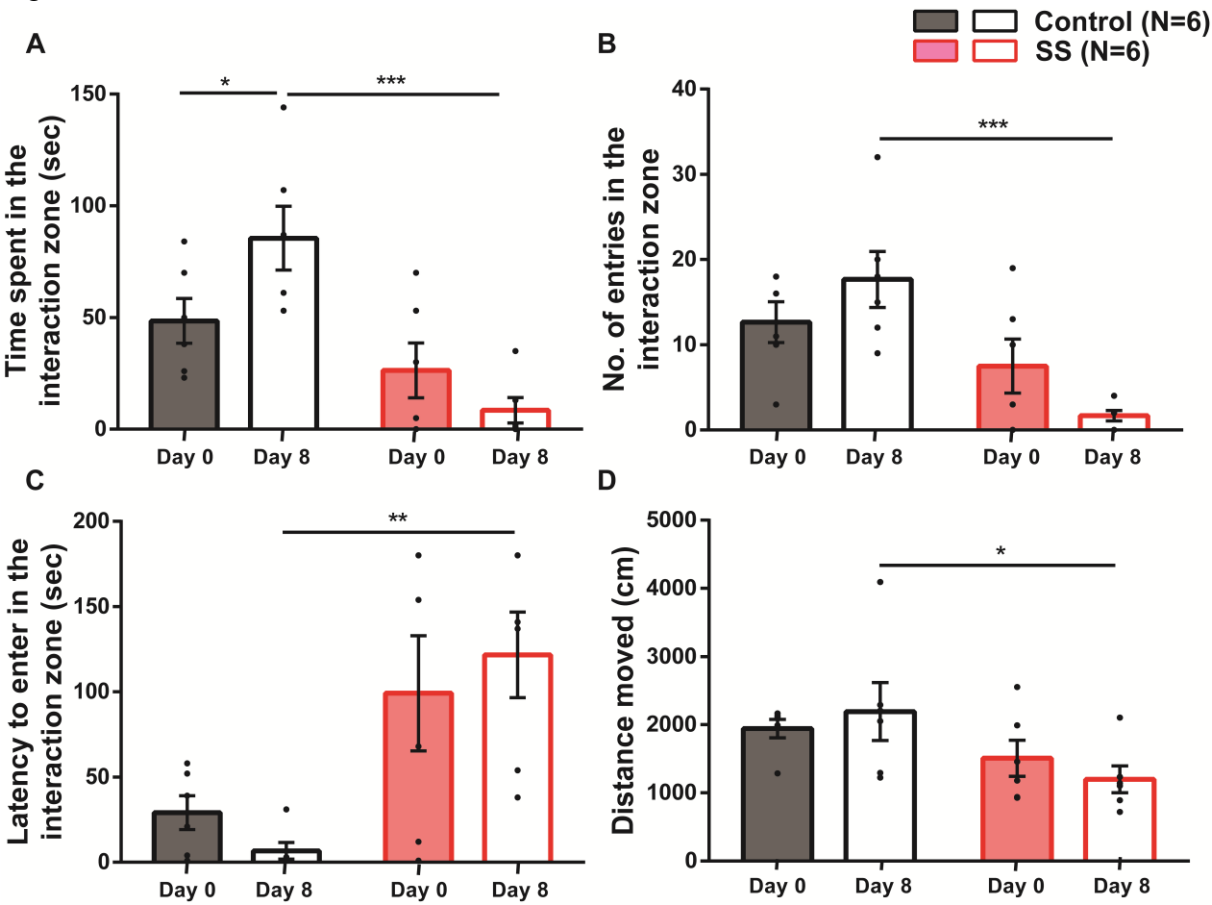
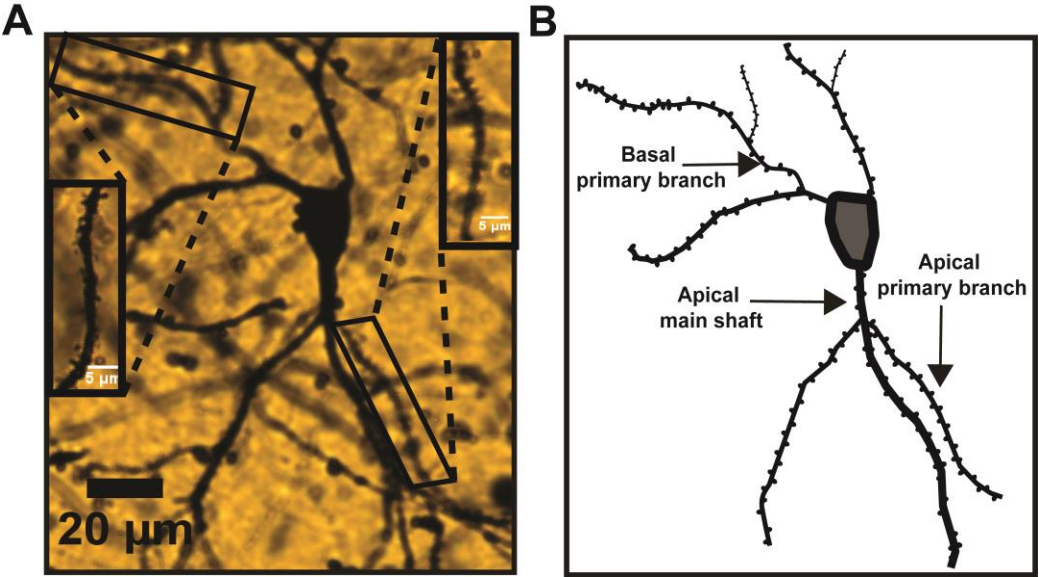
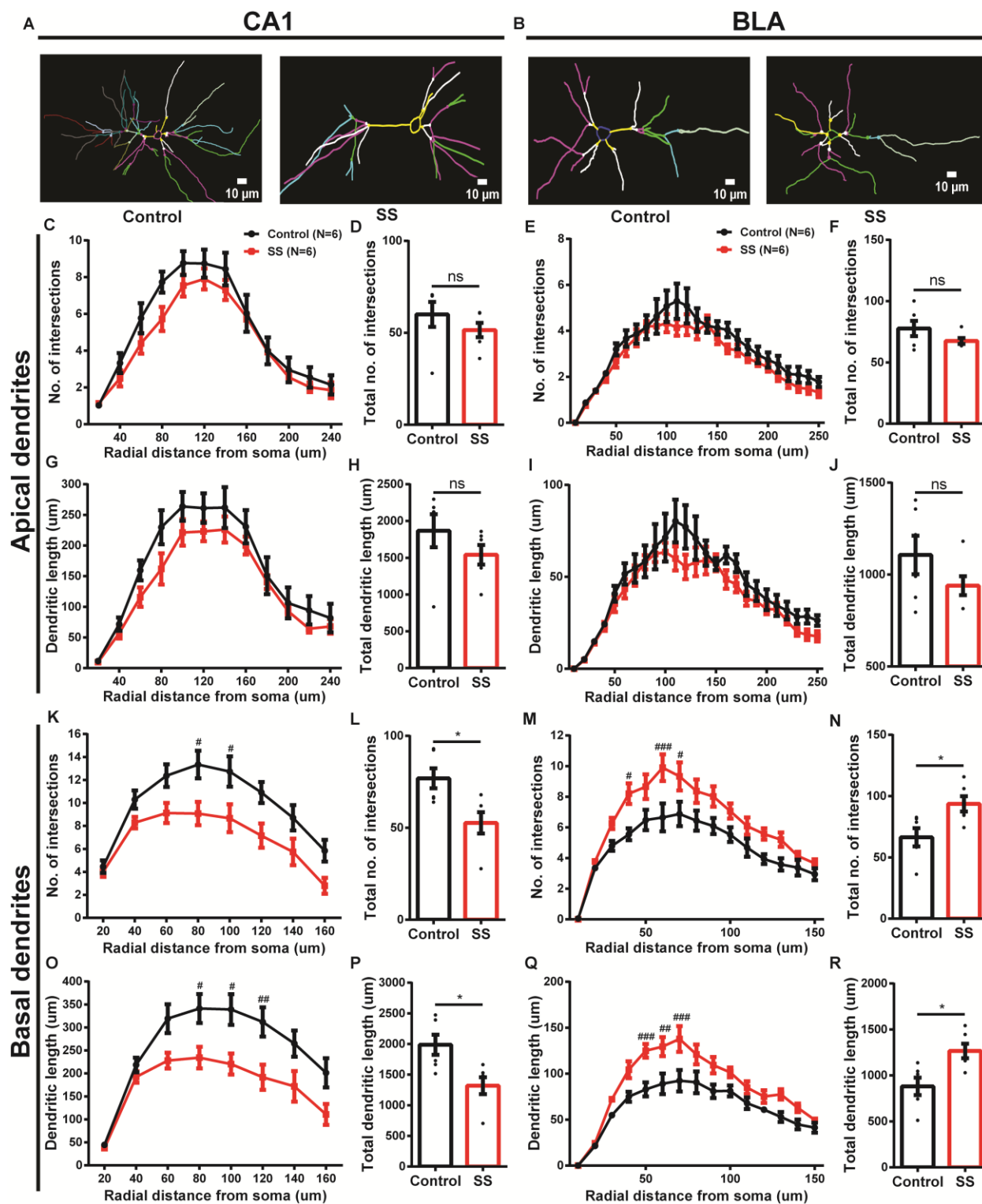
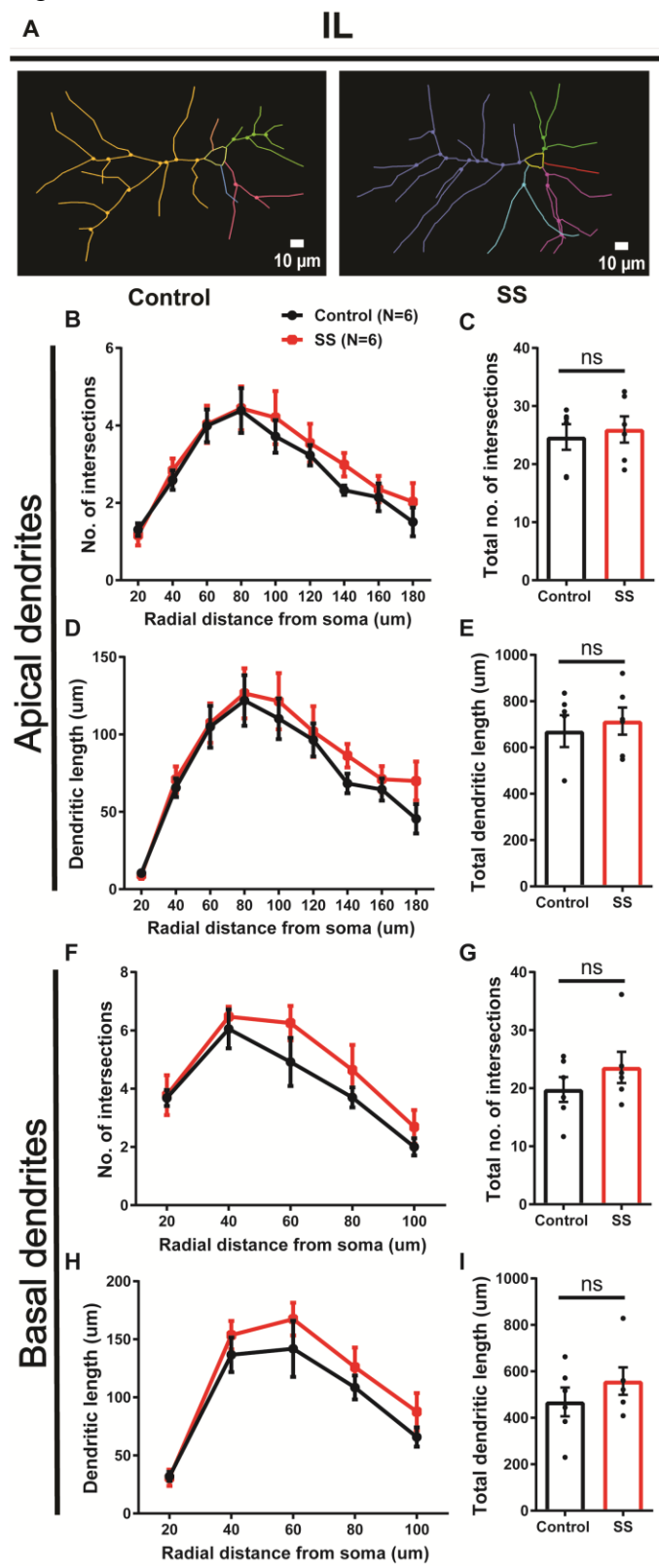
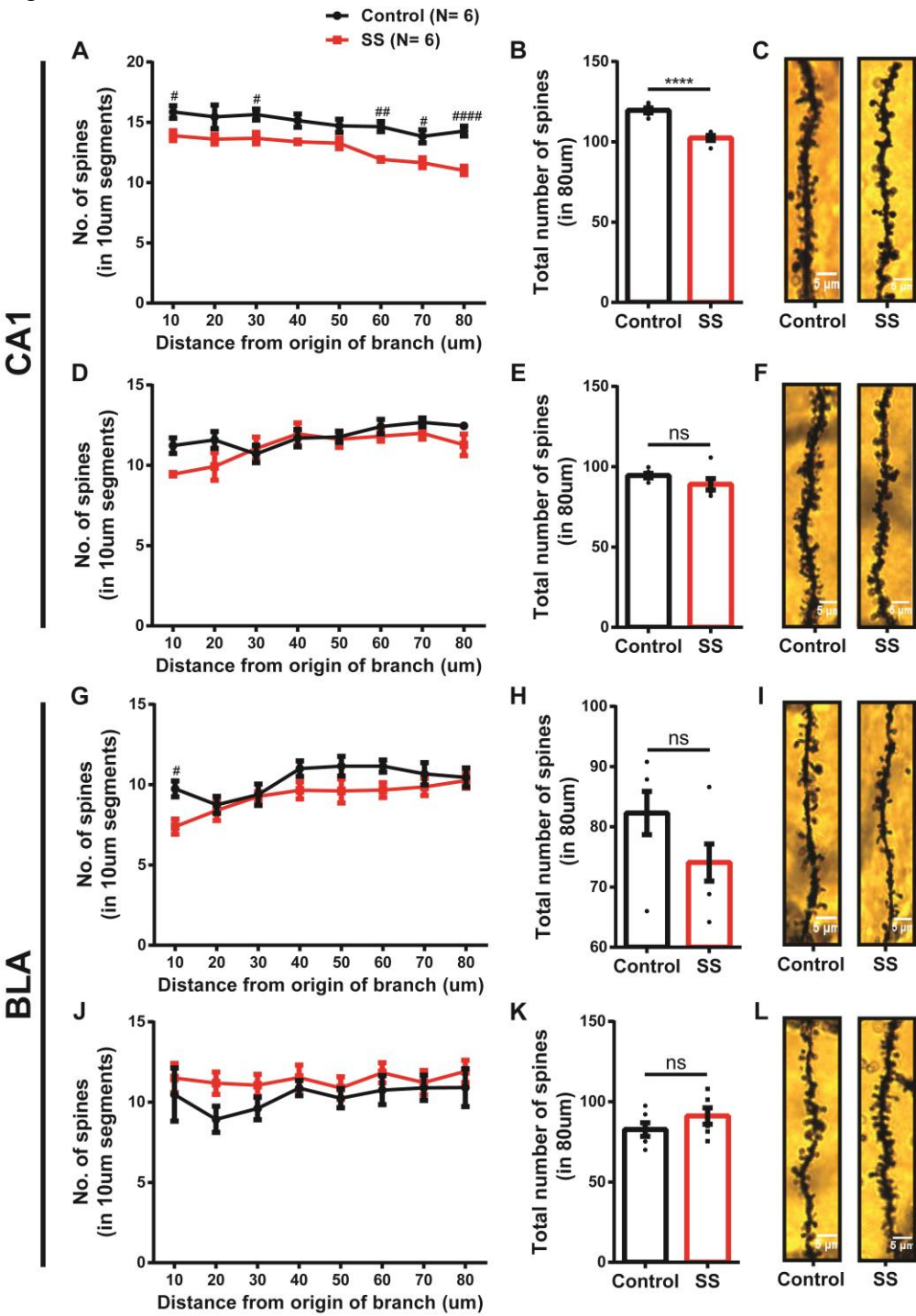


Fig. 4.

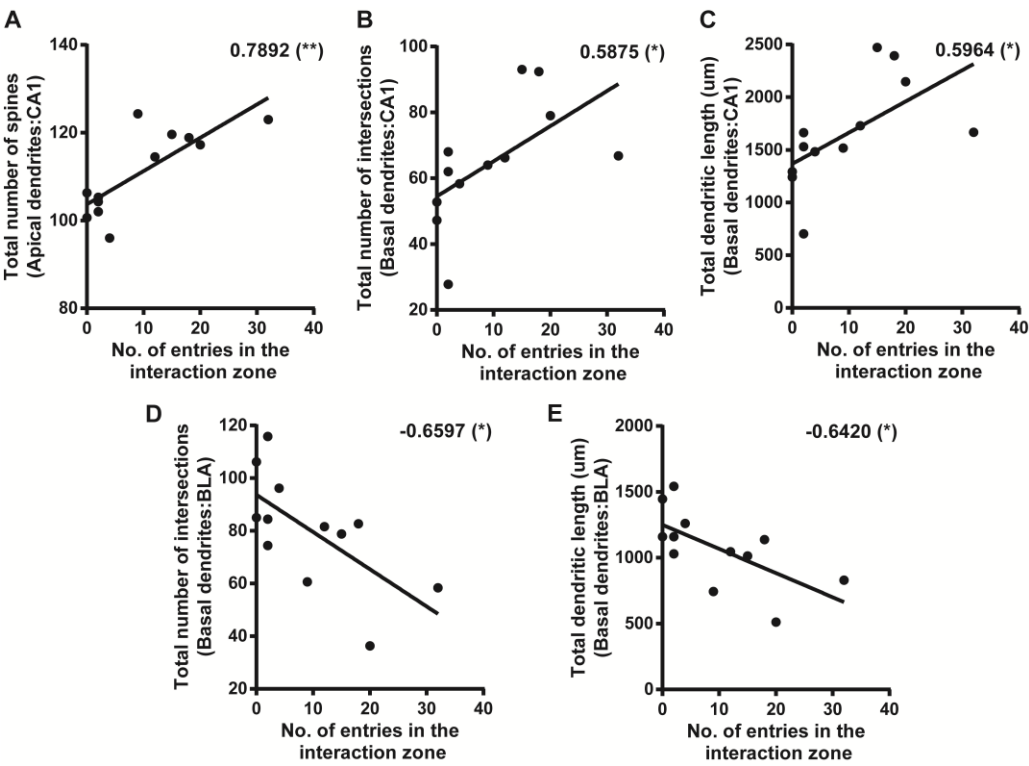








549 Fig. 8.
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